

# Localization of Thioredoxin in the Rat Brain and Functional Implications

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**The immunoreactivity for thioredoxin, which catalyzes protein disulfide reductions, has previously been shown to exist in nerve cells and their axons. Here we demonstrate the localization of thioredoxin mRNA as revealed by *in situ* hybridization in the rat brain. The gene is expressed in nerve cells of a variety of brain regions, for example, the cerebral cortex, the piriform cortex, the medial preoptic area, the CA3/CA4 region of the hippocampal formation, the dentate gyrus, the paraventricular nucleus of the hypothalamus, the arcuate nucleus, the substantia nigra pars compacta, the locus coeruleus, the ependyma of the 4th ventricle, and the epithelial cells of the choroid plexus. This distribution implicates an important function in nerve cell metabolism, especially in regions with high energy demands and indicates a role of the choroid plexus in nerve cell protection from environmental influences. It was found that after mechanical injury induced by partial unilateral hemitransection the thioredoxin mRNA expression is upregulated in the lesioned area and spreads to the cortical hemispheres at the lesioned level. This induction suggests a function of thioredoxin in the regeneration machinery of the brain following mechanical injury and oxidative stress.**

**[Key words: thioredoxin, mRNA, *in situ* hybridization, brain, rat, hemitransection]**

Thioredoxin is a multifunctional 12 kDa protein which catalyzes reductions of protein disulfides via an active site dithiol with the sequence -Cys-Gly-Pro-Cys-. As part of the thioredoxin system, that is, thioredoxin, NADPH, and FAD-containing enzyme thioredoxin reductase, thioredoxin is a hydrogen donor for ribonucleotide reductase, methionine sulfoxide reductases, or sulfate reductase. It is also a general protein disulfide reductase and regulates enzyme and protein activity by “thiol redox control” (Holmgren, 1985, 1989).

Thioredoxin has been isolated from a large variety of prokaryotic and eukaryotic species, making it ubiquitous (Holmgren

1985). In mammals, thioredoxin and thioredoxin reductase are widespread in different tissues and not only related to cellular growth and DNA synthesis (Holmgren, 1985; Hansson et al., 1986a,b). High levels of thioredoxin and thioredoxin reductase are present in nervous tissue, for example, in nerve cells and their axons (Rozell et al., 1985; Stemme et al., 1985). Thioredoxin is localized at intracellular membranes especially in secretory cells (Hansson et al., 1986a, 1989). Using antisera against rat thioredoxin the protein was observed to be widely distributed and its immunoreactivity is localized in the cytoplasm and enriched at plasma membranes or in the subplasma membrane zone. Thioredoxin levels vary in secretory cells dependent on their stage of metabolic activity (Hansson et al., 1986a, 1988, 1989). Recent results have identified thioredoxin as a secreted growth factor (Tagaya et al., 1989; Ericson et al., 1992) and a regulator of transcription factors (Matthews, 1992).

The cDNA encoding human thioredoxin has been cloned (Wollman et al., 1988; Tagaya et al., 1989; Tonissen and Wells, 1991). It is not known so far, in which nuclei of the mammalian brain the message is expressed and how this is related to functional aspects and electron transport to reductive enzymes. In the present article the first demonstration of the cellular localization and distribution of thioredoxin mRNA levels is given together with evidence for an involvement of thioredoxin in brain plasticity as seen from an upregulation of thioredoxin mRNA levels after a partial mesodiencephalic hemitransection.

## Materials and Methods

**Animals.** Male pathogen-free adult Sprague–Dawley rats (200 gm average body weight) were used for thioredoxin mRNA localization (four rats) and for thioredoxin mRNA localization after partial mesodiencephalic hemitransection (20 rats). They were kept under regular lighting conditions (lights on 6:00 A.M. and off 8:00 P.M.) at constant temperature (23°C) and had free access to food pellets and tap water.

**Partial meso-diencephalic hemitransection.** The anaesthetized rats (chloralhydrate 350 mg/kg body weight) were mounted in a stereotactic instrument (David Kopf, Tujunga, CA). After drilling out a circular-shaped piece of skull bone, the dura was penetrated with a rectangular knife (4 mm wide, 1 mm thick at the edge), which was tilted 20° to the frontal plane and inserted 1 mm caudal to bregma and 1 mm lateral to the midline (bregma -1.00 mm, L +1.00 mm; Paxinos and Watson, 1986). The knife was lowered 10 mm ventrocaudally into the right hemisphere. After touching the skull base it was withdrawn 2 mm, moved laterally until touching the bone and then removed.

The lesion cuts ascending and descending pathways including the nigrostriatal dopaminergic system with the exception of its most medial components (Agnati et al., 1983). As controls sham-operated rats were used (four rats). The rats were studied at several time-points after hemitransection: 24 hr, 72 hr, 7 d, 14 d (four rats in each group).

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The body weight of the hemitranssected rats decreased significantly 7 d after surgery as compared to sham operated rats and was still significantly less compared to sham operated rats at the 14 d time interval. However, at the 14 d time point the body weight was comparable to the basal value (Fuxe et al. 1990).

**Dissection and fixation.** For *in situ* hybridization studies the animals were anaesthetized with pentobarbital (60 mg/kg body weight) and transcardially perfused through the left cardiac ventricle with ice-cold 0.9% NaCl. The brain was removed and snap frozen in isopentane ( $-35^{\circ}\text{C}$ ). All material was sectioned at a 12  $\mu\text{m}$  thickness in a cryostat (Leica, Frigocut, Germany).

**DNA construct.** A human thioredoxin cDNA (open reading frame coding for 105 amino acid residues) (Tagaya et al., 1989) subcloned into plasmid pACA (Ren et al., 1993) was isolated by EcoRI digestion and subsequently subcloned into the EcoRI site of the pGEM 3Z vector. The orientation of the insert in different subclones was analyzed by nucleotide sequence analysis (Sanger et al., 1977) using T7 RNA polymerase (Promega, Madison, WI). Two subclones with opposite orientation were selected and digested with SmaI. Sense and antisense RNA were obtained using T7 RNA polymerase for *in vitro* transcription.

**RNA probe synthesis.** The probes were synthesized by *in vitro* transcription using the T7 RNA polymerase promoter for sense (s) and antisense (as) RNA production as well. For this purpose the constructs were cut with SmaI. The probes were synthesized according to the standard procedure described earlier (Bunnemann et al., 1992). Briefly,  $^{35}\text{S}$ - $\alpha$ -UTP (Du Pont NEN, Boston) was used to label the RNA transcripts in a mixture containing ribonucleotides, transcription buffer, RNase inhibitor (Boehringer Mannheim, Germany) and T7 RNA polymerase (Boehringer Mannheim, FRG) for 90 min at  $37^{\circ}\text{C}$ . Subsequently, the DNA template was removed by treatment with RNase free DNase I (Boehringer Mannheim, Germany) for 20 min at  $37^{\circ}\text{C}$ . The transcripts were purified chromatographically on NENSORB cartridges (Du Pont-NEN, Boston, MA) and checked by denaturing formaldehyde gel electrophoresis. The specific activity of the probes was in the order of  $5 \times 10^8$  dpm/ $\mu\text{g}$  RNA.

**In situ hybridization.** The procedure is described earlier in detail (Lippoldt et al., 1993). Briefly, the sections were brought to room temperature, fixed with 4% buffered paraformaldehyde, washed in PBS and deproteinized in 0.1 M HCl. After additional washing the sections were acetylated to avoid unspecific binding. After dehydration in graded ethanols the sections were prehybridized in a humidified chamber with 150 ml of prehybridization buffer [50% deionized formamide, 50 mM Tris-HCl pH 7.6, 25 mM EDTA pH 8.0, 20 mM NaCl, 0.25 mg/ml yeast tRNA,  $2.5 \times$  Denhardt's solution (0.05% Ficoll, 0.05% polyvinylpyrrolidone, 0.05% bovine serum albumin)] for 2–4 hr. After draining the prehybridization buffer off the slides, the sections were hybridized according to the standard procedure using 0.15 ng of labeled s and as RNA, respectively. The hybridization was done at  $37^{\circ}\text{C}$  in a buffer containing 50% formamide,  $1 \times$  Denhardt's solution, 10% dextran sulfate, 0.5 mg/ml yeast tRNA, 0.1 mg/ml poly-A, and 0.2 M DTT (for details, see Lippoldt et al., 1993) for 16–18 hr. Thereafter, the slides were washed in  $0.5 \times$  SSC/50% formamide at  $48^{\circ}\text{C}$  for 2 hr, 20 min in  $1 \times$  SSC at  $48^{\circ}\text{C}$  and subsequently treated with RNase A (10  $\mu\text{g}/\text{ml}$ ) for 30 min at  $37^{\circ}\text{C}$  and washed several times in  $1 \times$  SSC,  $0.5 \times$  SSC, and  $0.2 \times$  SSC at  $48^{\circ}\text{C}$ , dehydrated in graded ethanols, dried, and exposed on Hyperfilm- $^3\text{H}$  films (Amersham, UK) and coated with Kodak NTB2 emulsion, respectively. The slides were exposed at  $-20^{\circ}\text{C}$

for 3 weeks on the x-ray films and at  $4^{\circ}\text{C}$  for 6 weeks on emulsion. Emulsion coated slides were counterstained with cresyl violet.

The specificity of the method used was determined by hybridization with  $^{35}\text{S}$ - $\alpha$ -UTP labeled sense RNA at the same specific activity, length and concentration as the antisense RNA.

**Computer-assisted microdensitometry.** Semiquantitative data on the thioredoxin mRNA levels were obtained by measuring the gray values of the film autoradiograms using the microdensitometrical program of the IBAS 2.5 (Kontron, Germany) according to a previously published microdensitometric method (Zoli et al., 1990). Three measurements were performed for each region: (1) the total value, that is, measurements of the areas in question in the sections hybridized with  $^{35}\text{S}$ - $\alpha$ -UTP-labeled thioredoxin-antisense RNA; (2) the unspecific value, that is, measurements of the corresponding areas in the control sections hybridized with  $^{35}\text{S}$ - $\alpha$ -UTP-labeled thioredoxin-sense RNA; and (3) the background value, that is, measurements of the film background outside the sections. In order to obtain a rostrocaudal evaluation of the labeling intensity, the analyses were performed at various coronal levels. The autoradiograms were digitized directly via a TV camera (CCD 72/MTi-camera) on a screen, allowing the measurement of an entire coronal section at once. The areas of interest were selected by means of a light pen and the transmissions were measured. The specific values were defined as those obtained by subtracting the nonspecific values (sections incubated with the labeled sense RNA-probe) from the total values (sections incubated with labeled antisense RNA-probe) after correcting for the background. Photographs of the *in situ* autoradiograms were directly taken from the x-ray film. Transmissions as well as optical densities were obtained utilizing postprocessing programs.

The measurement for the different regions was done as illustrated in Figure 4a for the cortical parts at all time points. Since at the 24 hr time interval the tissue within the lesioned area is deteriorated the expression within the lesioned area was measured in sections where some of the tissue was left. At the other time points the lesion was encircled and measured. The sense values were in all cases background values. Pictures have been taken directly from the x-ray film.

For statistical analysis multiple *t* test was used. As a basis for the statistical tests served the mean values of the measured specific transmissions and their standard deviations.

## Results

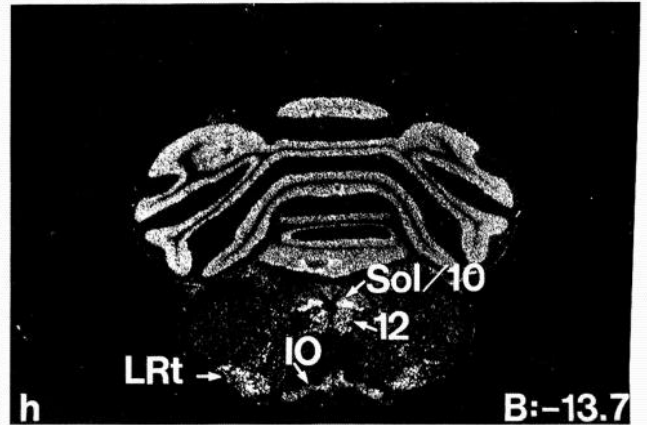
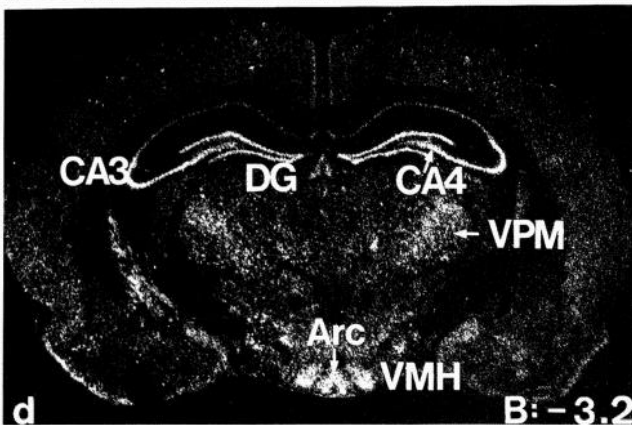
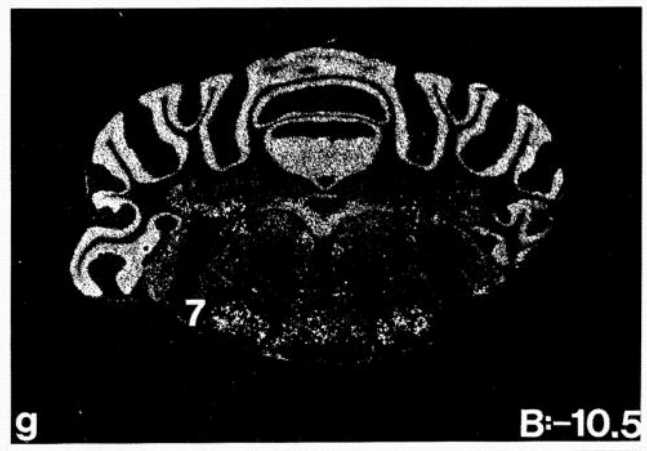
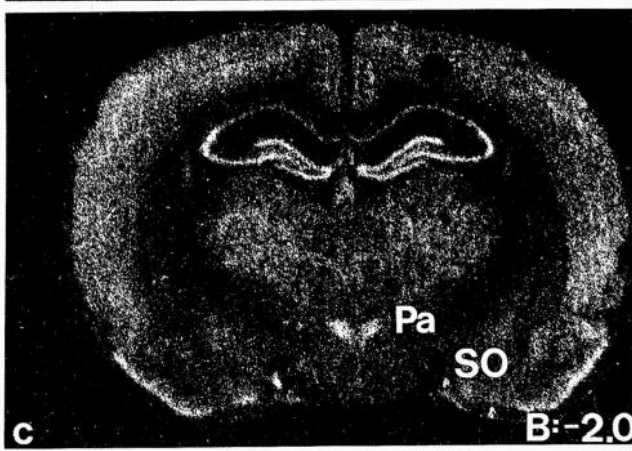
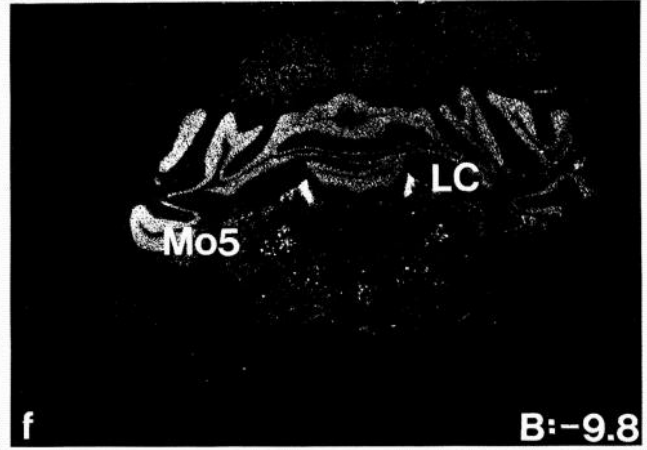
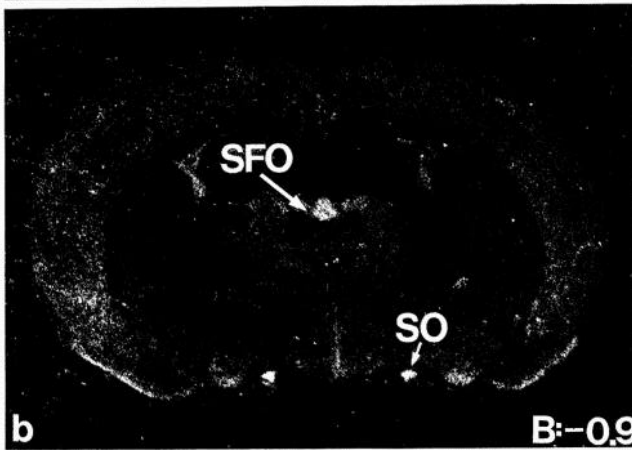
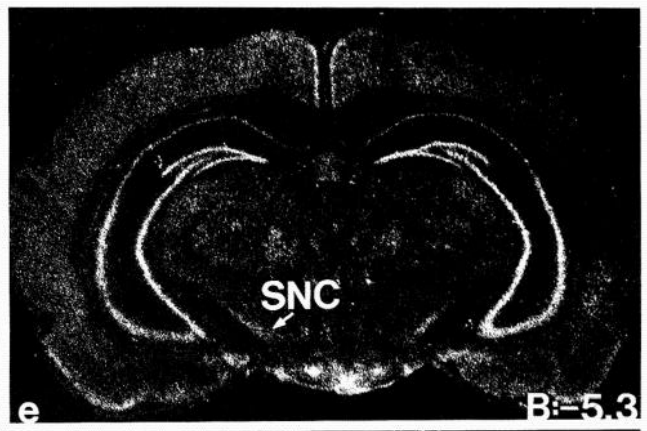
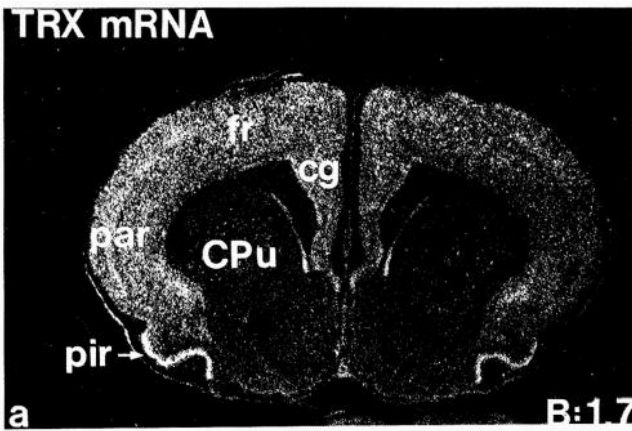
### Distribution of the thioredoxin mRNA in the rat brain

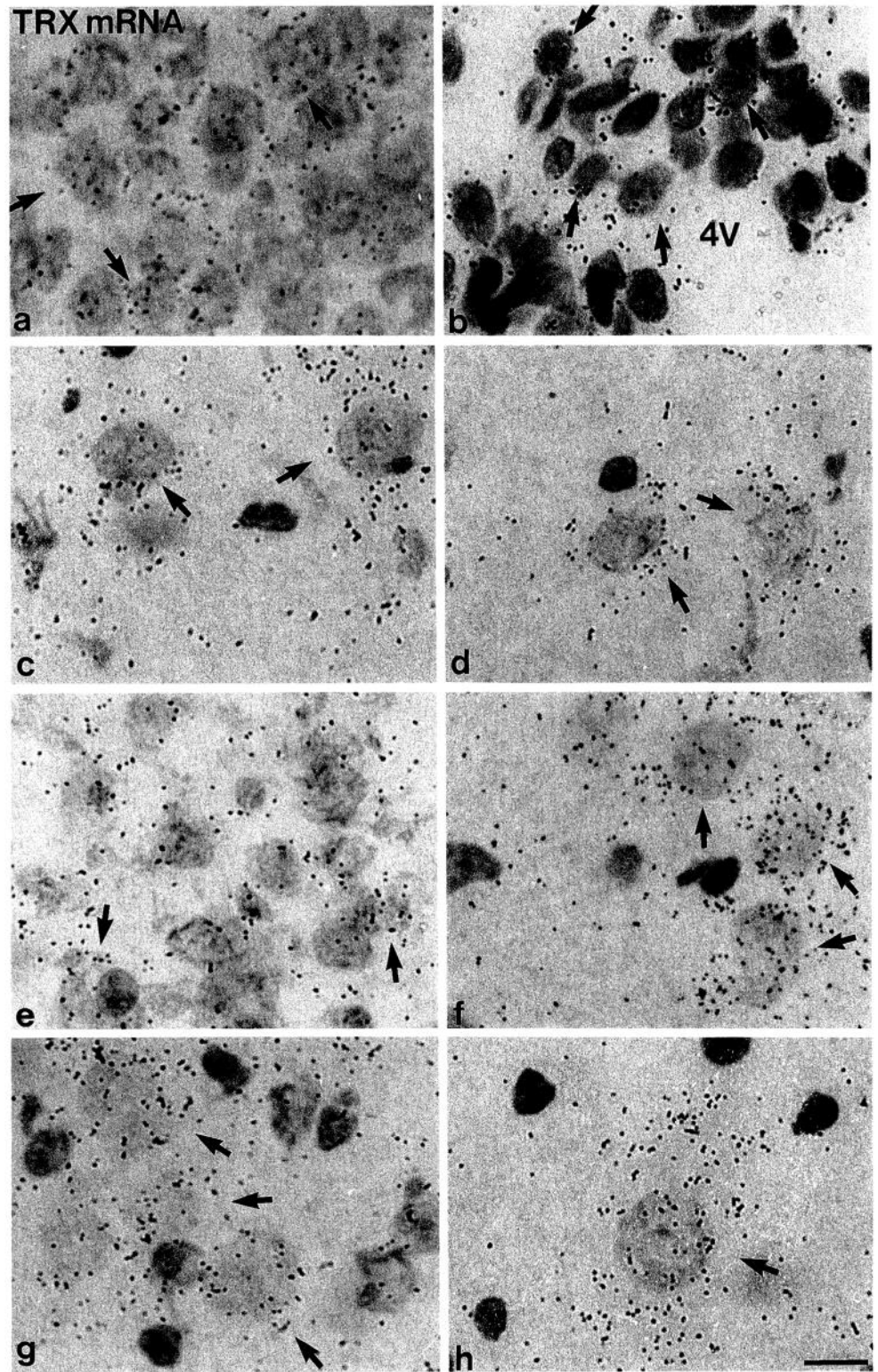
The thioredoxin mRNA is widely distributed throughout the rat brain. In all areas investigated the thioredoxin mRNA is expressed in nerve cells (Fig. 2).

**Telencephalon.** High expression can be seen in the cortex from the frontal to the occipital level. This expression is localized in the cingulate cortex, the frontal cortex, the parietal cortex, the temporal cortex, the piriform cortex, the entorhinal cortex and the amygdala. There is no significant difference in optical density from bregma 4.20 mm to bregma  $-0.40$  mm (Fig. 1a). The highest expression shows the piriform cortex, especially at bregma 3.20 mm to  $-0.40$  mm (Fig. 1a). This labeling was due to expression of the gene in the granule cells. There was no variation in rostro-caudal direction as seen by measure-

**Figure 1.** Thioredoxin mRNA distribution in selected regions of the rat brain. *a*, Thioredoxin gene expression in the rat cerebral cortex. *cg*, Cingulate cortex; *fr*, frontal cortex; *par*, parietal cortex. In the piriform cortex (*pir*) the gene is very highly expressed whereas in the caudate putamen (*CPu*) the expression is only slightly above background level (bregma, 1.7 mm). *b*, The subfornical organ (*SFO*) and the supraoptic nucleus (*SO*) are moderately expressing thioredoxin mRNA (bregma,  $-0.9$  mm). *c*, The paraventricular hypothalamic nucleus (*Pa*) is one of the nuclei expressing the highest amounts of thioredoxin mRNA. The rostral part of the supraoptic nucleus (*SO*) is labeled by thioredoxin mRNA (bregma,  $-2.0$  mm). *d*, In the CA3–CA4 region of the hippocampal formation and the dentate gyrus (*DG*) the thioredoxin gene is highly expressed, whereas the CA1 and CA2 region shows very low gene expression. Within the thalamus the gene is moderately expressed in the ventroposteromedial thalamic nucleus (*VPM*), in the hypothalamus the ventromedial hypothalamic nucleus (*VMH*) and the arcuate nucleus (*Arc*) are strongly labeled by the thioredoxin mRNA probe (bregma,  $-3.2$  mm). *e*, The substantia nigra pars compacta (*SNC*) is moderately positive for thioredoxin mRNA (bregma,  $-5.3$  mm). *f*, The locus coeruleus (*LC*) is one of the regions with the highest thioredoxin mRNA expression. *Mo5*, Motor trigeminal nucleus (bregma,  $-9.8$  mm). *g*, At bregma  $-10.5$  mm the facial nucleus (*7*) is strongly positive for thioredoxin mRNA. *h*, The medulla oblongata exhibits structures moderately and highly expressing the thioredoxin gene, that is, the nucleus of the solitary tract (*Sol*), the dorsal motor nucleus of the vagus (*10*), the hypoglossal nucleus (*12*) and in the medial part as well as the lateral parts of the reticular formation (*LRt*) and the inferior olive (*IO*) (bregma,  $-13.7$  mm).

TRX mRNA





**Figure 2.** Cellular localization of thioredoxin mRNA using emulsion and counterstaining with cresyl violet. *a*, In the piriform cortex thioredoxin mRNA signals are seen in the densely packed nerve cells (arrows). *b*, A patchy distribution of the signals for thioredoxin mRNA in the epithelial cells of the choroid plexus in the 4th ventricle (4V) is seen (arrows). *c*, Within the paraventricular hypothalamic nucleus the thioredoxin gene is expressed in nerve cell bodies (arrows). *d*, In the ventral posterolateral thalamic nucleus the grains are localized over the big nerve cells (arrows). *e*, In the CA3 region of the hippocampal formation the grains are localized over the nerve cell nuclei (arrows). *f*, In the inferior olive the gene is expressed in large nerve cell bodies (arrows). *g*, The big nerve cells of the locus coeruleus contain the signals for thioredoxin mRNA (arrows). *h*, A big nerve cell of the nucleus tractus solitarius exhibiting the mRNA signal for thioredoxin (arrow), small glial cell nuclei do not express thioredoxin (arrowhead). Scale bar, 10  $\mu$ m.

ments between bregma 3.20 mm to bregma  $-0.40$  mm (Fig. 1*a*). The caudate putamen shows very low expression of the message, whereas the ependymal cells of the lateral ventricle express thioredoxin (Fig. 1*a*) (Table 1).

In the *hippocampal formation* the thioredoxin gene is mainly expressed in the nerve cells of the CA3 region but also in the CA4 region and the granule cells of the dentate gyrus. All

regions show comparable optical densities. The measurements are made at bregma  $-2.80$  mm to  $-3.00$  mm (Fig. 1*b*). Very low amounts of thioredoxin mRNA expression have been observed in the CA1 region (Fig. 1*b*).

The *basal ganglia* including caudate putamen, globus pallidus and ventral pallidum show expression of the thioredoxin gene only slightly above background. Structures related to basal gan-



**Table 1. Distribution of thioredoxin mRNA levels in the rat brain as determined with microdensitometry**

Area	Optical density	
	Mean $\pm$ SD	<i>n</i>
<b>Telencephalon</b>		
Cerebral Cortex		
Cingulate cortex	0.104 $\pm$ 0.018	5
Frontoparietal cortex	0.090 $\pm$ 0.019	5
Piriform cortex	0.129 $\pm$ 0.038	5
<b>Basal ganglia</b>		
Caudate putamen	0.049 $\pm$ 0.026	3
<b>Preoptic region</b>		
Medial preoptic area	0.141 $\pm$ 0.027	2
Magnocellular preoptic nuc.	0.101 $\pm$ 0.018	1
<b>Diencephalon</b>		
<b>Hypothalamus</b>		
Supraoptic nucleus	0.123 $\pm$ 0.046	1
Paraventricular hypothalamic nucleus	0.337 $\pm$ 0.076	1
Arcuate nucleus	0.224 $\pm$ 0.052	2
Ventromedial hypothalamic nucleus	0.164 $\pm$ 0.042	3
<b>Thalamus</b>		
Medial habenula	0.163 $\pm$ 0.023	2
Subthalamic nucleus	0.205 $\pm$ 0.099	1
Ventral posteriolat./posteriomed. thal. nuc.	0.113 $\pm$ 0.023	2
Ventrolat/Ventromed. thal. nuc.	0.111 $\pm$ 0.021	2
<b>Mesencephalon</b>		
Substantia nigra pars compacta	0.115 $\pm$ 0.058	1
<b>Pons</b>		
Locus coeruleus	0.379 $\pm$ 0.123	2
Pontine nuclei	0.232 $\pm$ 0.084	2
Motor trigeminal nuc.	0.168 $\pm$ 0.045	2
<b>Medulla oblongata</b>		
Inferior olive	0.252 $\pm$ 0.080	3
Nucleus of the solitary tract	0.384 $\pm$ 0.113	2
Hypoglossal nucleus	0.285 $\pm$ 0.044	2
Fascial nucleus	0.235 $\pm$ 0.037	2
Lateral reticular nuc.	0.178 $\pm$ 0.030	3
Choroid plexus (4th ventricle)	0.126 $\pm$ 0.038	2
<b>Circumventricular organs</b>		
Subfornical organ	0.148 $\pm$ 0.046	1
<b>Hippocampal formation</b>		
CA1	0.053 $\pm$ 0.013	2
CA3/CA4	0.149 $\pm$ 0.040	3
Dentate gyrus	0.152 $\pm$ 0.051	3

*n*, Number of rostro-caudal levels examined. Means  $\pm$  SD are shown out of four rats. In each rat and each area at least three sections were measured.

glia with regard to function such as the *substantia nigra* and the *subthalamic nucleus* present more intense labeling. Substantia nigra is only labeled within the pars compacta as illustrated at bregma  $-4.80$  mm to  $-5.00$  mm (Fig. 1c) and shows less labeling than the subthalamic nucleus at bregma  $-3.80$  mm to  $-4.16$  mm. The *magnocellular preoptic nucleus* as well as the *nucleus of the horizontal limb of the diagonal band* shows a moderate expression of the message.

**Diencephalon.** The thioredoxin mRNA was moderately expressed within all the preoptic nuclei. Thalamic and hypothalamic regions contain moderate to high amounts of thioredoxin mRNA. Highest expression levels were observed in the para-

ventricular hypothalamic nucleus (Fig. 1f), the arcuate nucleus (Fig. 1b) and the subthalamic nucleus. Other regions like the supraoptic nucleus (Fig. 1f), the ventromedial hypothalamic nucleus (Fig. 1b), the medial habenula, the ventralposteriolateral/posteriomedial thalamic nucleus (Fig. 1b) and the ventrolateral/ventromedial thalamic nucleus were moderately labeled.

**Pons.** The *pontine nuclei* (bregma:  $-6.80$  mm to  $-7.00$  mm) are among the most intensely labeled structures in the rat brain. Moreover, the *locus coeruleus* (bregma:  $-10.04$  mm to  $-9.7$  mm) is the structure with the most intense labeling among all structures measured (Fig. 1h) and the *motor nucleus of the trigeminal nerve* (bregma:  $-9.7$  mm) shows moderate expression of the thioredoxin gene (Fig. 1h).

**Medulla oblongata.** The lateral *reticular nucleus* (LRt) (Fig. 1d,g) (bregma:  $-13.7$  mm), the *nucleus of the solitary tract* (NTS) (lateral and medial part, bregma:  $-13.8$  mm to  $-13.6$  mm), the *fascial nucleus* (bregma:  $-10.5$  mm to  $-11.0$  mm) and the *inferior olive* (IOC) (bregma:  $-13.7$  mm) were the most intensely labeled structures in the medulla oblongata (Fig. 1d). Within the medulla oblongata, the NTS shows strong gene expression, whereas the IOC, fascial nucleus, and the LRt were moderately labeled.

The *4th ventricle choroid plexus* (bregma:  $-13.2$  mm) shows moderate thioredoxin mRNA expression.

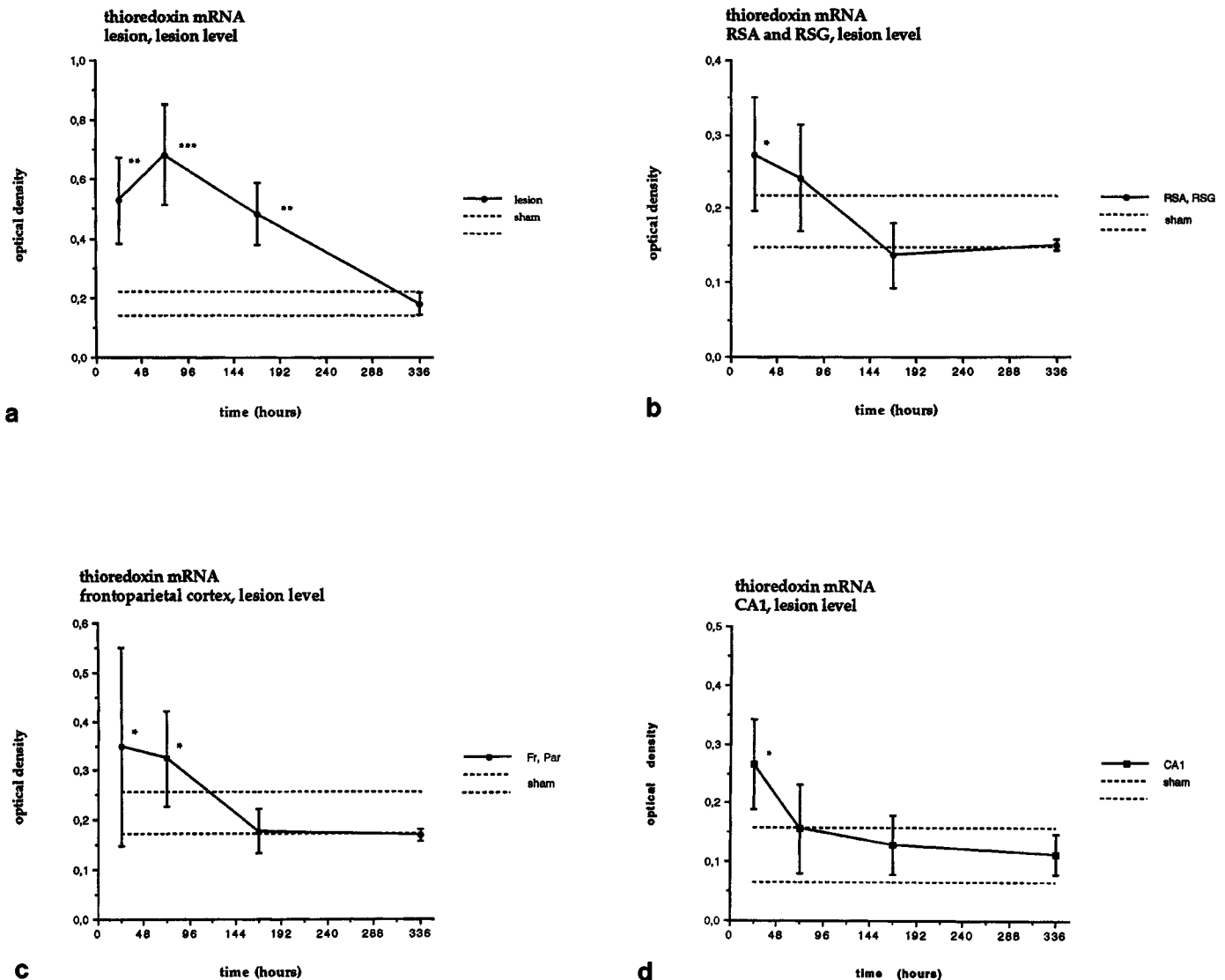
**Circumventricular organs.** The *subfornical organ* (bregma:  $-1.4$  mm) of the rat brain is moderately labeled (Fig. 1e), whereas no clear signal is observed in the median eminence and the area *prostroma*.

#### Partial meso-diencephalic hemitranssection

The thioredoxin mRNA levels are highly upregulated at 24 hr around the lesion site and also in the surrounding cortical hemisphere (retrosplenial and frontoparietal cortices) of the ipsilateral side. After 72 hr the increased mRNA signal is more restricted to the lesion site and only the frontoparietal part of the cerebral cortex is still activated. At the 7 d time interval the expression of the thioredoxin gene within the cortical areas is almost normal. However, the cells within the lesioned area still show an increased labeling. Fourteen days after the surgery the thioredoxin mRNA levels are normalized (Figs. 3, 4). The CA1 region of the hippocampal formation exhibits a higher level of thioredoxin mRNA expression 24 hr after injury. This expression is back to normal control values after 72 hr.

#### Discussion

Thioredoxin mRNA levels are widely distributed throughout the rat brain. The highest expression levels have been observed in the piriform cortex, the dentate gyrus, the CA3/CA4 region of the hippocampal formation, the locus coeruleus as well as in some regions involved in neuroendocrine and/or cardiovascular control such as the paraventricular hypothalamic nucleus and the nucleus of the solitary tract. There is little known until now about the functional importance of thioredoxin gene expression in these regions. Since thioredoxin is a general protein disulfide oxidoreductase (Holmgren, 1979) and may regulate protein activity by "thiol-redox control" (Holmgren, 1985, 1989) it should have functions in signal transduction. Indeed, Deiss et al. (1991) cloned the thioredoxin gene, when the signal transduction pathway for interferon was analyzed by genetic techniques. It is interesting to note that thioredoxin expression is particularly abundant in regions with high energy demands or high activity involving redox reactive metabolites such as the substantia nigra



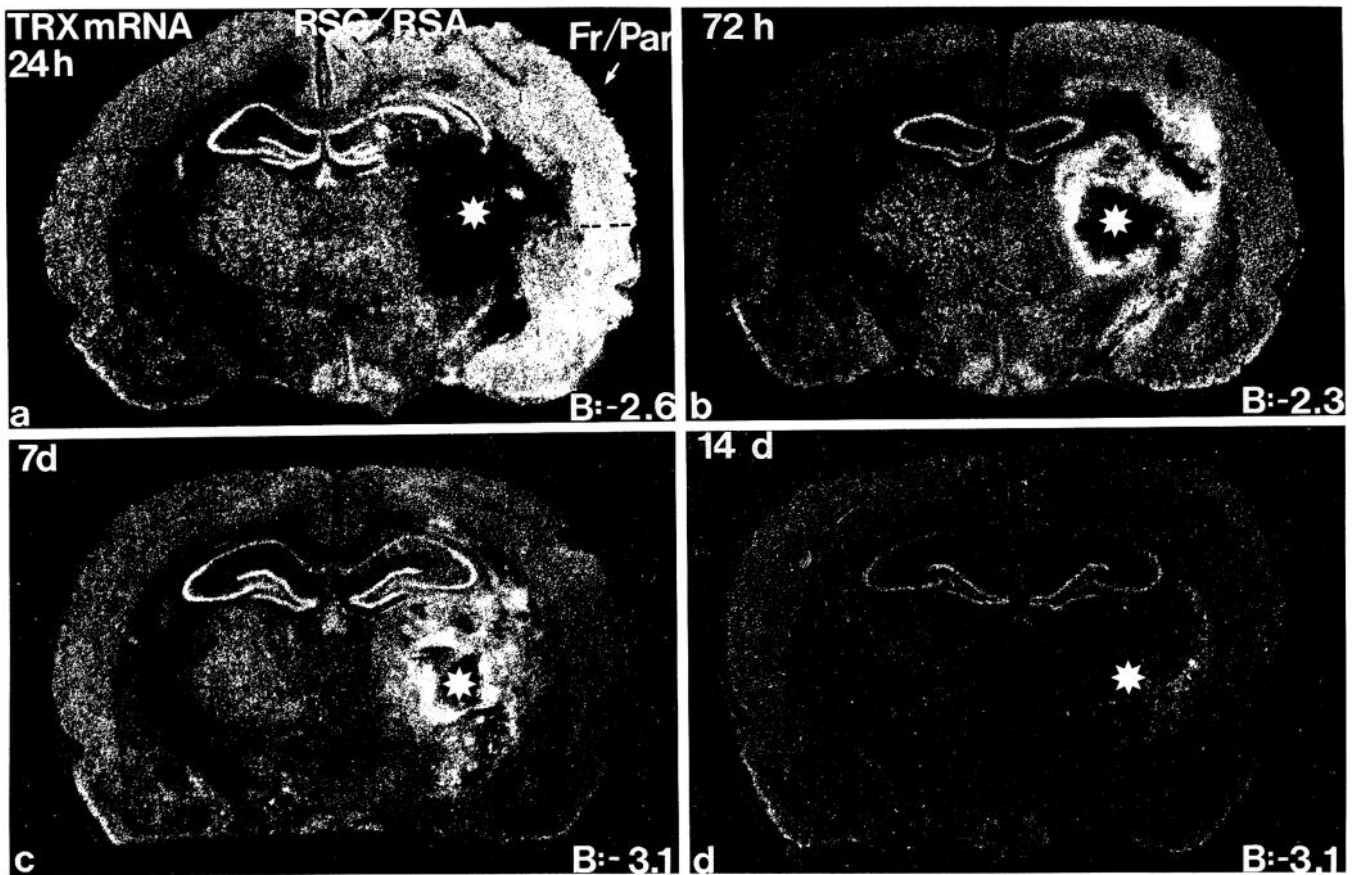
**Figure 3.** Time-dependent expression of thioredoxin mRNA after unilateral partial hemitransection. The thioredoxin mRNA levels of sham operated animals (*sham*) were expressed as optical densities  $\pm$  SD (shown as *parallel dotted lines*). The thioredoxin mRNA expression after hemitransection was expressed as optical densities (means  $\pm$  SD,  $n = 4$ ) ( $\bullet$ ). *a*, Lesioned area (bregma:  $-2.3$  mm to  $-3.1$  mm); *b*, retrosplenial agranular cortex (RSA) and retrosplenial granular cortex (RSG) (bregma:  $-2.3$  mm to  $-3.1$  mm); and *c*, frontoparietal cortex (*Fr, Par*) (bregma:  $-2.3$  mm to  $-3.1$  mm). *d*, CA1 region of the hippocampal formation (bregma:  $-2.3$  mm to  $-3.1$  mm). The measurement for the different regions was undertaken as shown in Figure 4a. Statistical analysis was made using multiple *t* test (\*\*\*,  $p < 0.001$ ; \*\*,  $p < 0.01$ ; \*,  $p < 0.05$ ).

and the subthalamic nucleus. Roles in keeping enzymes active in the presence of oxidizing redox active substrates or a direct role in regulating receptor and enzyme function is suggested. This would include information handling in the normal uninjured brain. In contrast, the CA1 region of the hippocampal formation, very well known for the vulnerability of its neurons to oxidative stress (Leifer and Kowall, 1993; Ordy et al., 1993; Neumann-Haefelin et al., 1994), is characterized by very low thioredoxin expression. This could be in part the reason for its vulnerability to situations like ischemia. Indeed, it has been shown, that glutathione is essential for hippocampal neurons to recover from damage by free radicals (Pellmar et al., 1992).

Previously, the calf brain was found to contain thioredoxin (0.05% of wet weight) determined by radioimmunoassay (Holmgren and Luthman, 1978). Immunohistochemical investigations showed thioredoxin and thioredoxin reductase to be present in the nerve cells in the rat brain (Rozell et al., 1985). Furthermore,

thioredoxin and thioredoxin reductase were found to be transported by axoplasmic transport in the rat sciatic nerve (Stemme et al., 1985a). These results and the present investigation with localization of thioredoxin mRNA give evidence for the continued synthesis of this protein by the nerve cells themselves. Indeed, since thioredoxin has been shown to be secreted from human B-cells and several other cell types (Ericson et al., 1992; Sitia et al., 1992), secretion from nerve cells may help to protect cells via anti-oxidative roles of thioredoxin together with glutathione peroxidase. It was recently shown (Björnstedt et al., 1994) that the thioredoxin and glutaredoxin systems are efficient electron donors to human plasma glutathione peroxidase, which operates in serum devoid of glutathione.

The present results demonstrate thioredoxin mRNA levels in ependymal cells of the 3rd ventricle and in epithelial cells of the choroid plexus as well. Here a patchy pattern of gene expression was evident. Thioredoxin immunoreactivity has previously been



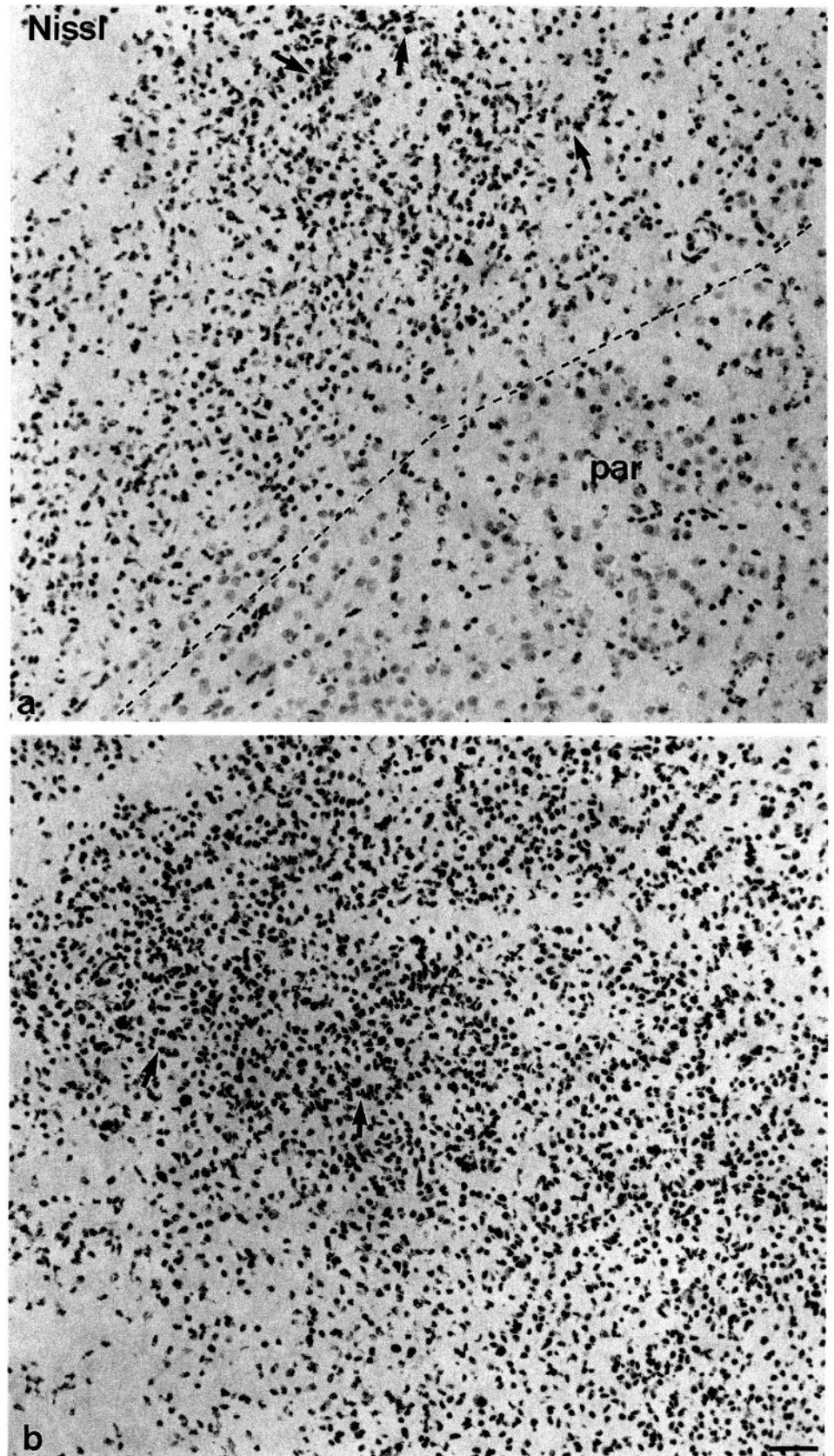
**Figure 4.** Illustration of the upregulation of thiodoxin mRNA expression after unilateral partial hemitransection as revealed by *in situ* hybridization. *a*, Thiodoxin mRNA 24 hr after injury, RSG/RSA-retrosplinal granular and agranular cortices, Fr/Par-fronto-parietal cortex. The white dotted line shows the ventral border between RSA/RSG and Fr, and the black dotted line shows the border for measurement of the fronto-parietal cortex. *b* illustrates the thiodoxin gene expression 72 hr after the injury; *c* demonstrates the thiodoxin mRNA after 7 d. The area surrounding the lesion is strong labeled for thiodoxin mRNA; *d*, 14 d after the lesion. The thiodoxin mRNA levels have become normalized (\*, lesion site).

found in many epithelial cells (Rozell et al., 1985; Hansson et al., 1988; Padilla et al., 1992) and in the secreting cells of the choroid plexus as well (Rozell et al., 1984). The function of thiodoxin expressed in the epithelial cells of the choroid plexus and ependymal cells of the ventricle may be related to their cell metabolism itself. Additionally, the secretion of thiodoxin into the cerebrospinal fluid may lead to the protection of nerve cells from oxidation by environmental influences like reactive oxygen intermediates via bulk flow into the extracellular fluid, thus maintaining the microenvironment of the brain cells (Nilsson et al., 1992). Moreover, thiodoxin could directly influence the secretory state of the epithelial cells of the choroid plexus and the ependymal cells of the ventricle, probably via control of cytoplasmic redoxbalance, as has been shown for the epithelial cells of the gastric mucosa and exocrine and endocrine pancreas (Hansson et al., 1986, 1988). It may also have a function in supporting the transport of substances from the blood to the CSF and vice versa by influencing the redox state of the transporter systems in the choroid plexus (Ericson et al. 1992).

Thiodoxin mRNA was also detected in the nigral dopaminergic cells of the zona compacta, which may give it a putative role in Parkinson's disease as suggested previously by Endoh et al. (1993).

Under pathophysiological conditions such as head trauma (mechanical injury), it is shown in the present article that the

expression of the thiodoxin gene is strongly induced in the lesioned area reaching into cortical regions of the ipsilateral hemisphere. The CA1 region of the hippocampal formation shows only a slight transient upregulation of the thiodoxin mRNA after the lesion. However, the cellular location of this increased thiodoxin mRNA signal still remains to be determined, but it may be linked to activated glial cells since the region surrounding the lesion is filled up with activated astroglial and microglial cells. However, in the uninjured brain the glial cells have been shown to lack thiodoxin immunoreactivity (Rozell et al., 1985; Hansson et al., 1989). The thiodoxin mRNA induction is an early event and occurs already 24 hr after injury. Since reactive oxygen intermediates play a crucial role in tissue damage after injury, the upregulation of the thiodoxin gene expression may be due to the involvement of the thiodoxin in protecting the lesioned area from active oxygen intermediates. As a result of such active oxygen intermediates, the methionine residues may be oxidized to methionine sulphoxide residues in proteins and thiodoxin together with methionine sulphoxide reductases will repair such proteins. Furthermore, many proteins will contain artificial disulfide bonds which may be reduced by thiodoxin reductase NADPH and thiodoxin. It appears that thiodoxin mRNA is induced following some signalling on the gene level involving increased oxidative stress via an as yet unknown mechanism. Particularly, interesting has



**Figure 5.** Cresyl violet staining of the hemitranssected region 7 d after the partial unilateral hemitranssection. *a*, Morphology of the lesion near the parietal cortex (*par*). The lesioned region (on the left-hand side of the *dotted line*) demonstrates densely packed small cell nuclei probably related to different types of glial cells (*arrows*). *b*, Densely packed small glial cell nuclei are characteristic for the tissue repair via gliosis within the lesioned area (*arrows*). Scale bar, 50  $\mu$ m.



been the information in recent years that thioredoxin is involved in the regulation of a number of transcription factors including NF- $\kappa$ B, AP-1, TF3C, TZLF1, and myb. In the case of NF- $\kappa$ B, it has been shown both *in vitro* and *in vivo* that human thioredoxin is responsible for the activation of the DNA binding properties by specifically reducing a disulfide bond involving a critical cysteine residue at position 62 in the P50 subunit (Abate et al., 1990; Matthews et al., 1992; Hayashi et al., 1993; Mitomo et al., 1994). The slight and transient increase in thioredoxin mRNA in the CA1 subfield of the hippocampus after the lesion may be one reason for its high vulnerability to increased oxidative stress and to the late onset of neuronal activation as seen by c-fos expression (Neumann-Haefelin et al. 1994).

It should also be noted that thioredoxin has been identified as a highly expressed cytokine-like factor in activated T- and B-cells which upregulates the interleukin-2 receptor  $\alpha$ -chain and interleukin-2, both of which are under the transcriptional control of NF- $\kappa$ B (Tagaya et al., 1989; Wakasugi et al., 1990). A protective role for damaged neuronal cells may thus be envisioned for thioredoxin secreted from glial cells or in particular from microglia/macrophages in the damaged ipsilateral area. Direct involvement of thioredoxin in transcriptional control activity with a variety of genes in the injured area in activated cells is a likely result. Repair mechanisms would also require DNA synthesis and thioredoxin is a hydrogen donor for ribonucleotide reductase and a redox regulator of the enzyme. In the case of injury, it may be able to supply the deoxyribonucleotides for DNA synthesis in dividing glial cells and channel nucleotides for repair in nerve cells.

Several recent reports suggest that thioredoxin may have important, but as yet undefined co-cytokine activities under pathophysiological conditions involving oxidative stress. This includes observations with hydrogen peroxide (Spector et al., 1988) as well as with TNF- $\alpha$  (Matsuda et al., 1991) or experimental ischemia (Endoh et al., 1993; Tomimoto et al., 1993). Thioredoxin has also been shown to modify the glucocorticoid receptor to a steroid binding site by reduction (Grippio et al., 1985). A specific role for thioredoxin in reticular site initiation of protein synthesis has been shown (Holmgren, 1985).

In conclusion, the thioredoxin gene expression has been shown to be widely distributed but preferentially located in distinct nerve cell populations in the rat brain. It is also strongly induced under pathophysiological conditions such as mechanical injury. Thus, one role of thioredoxin may be to form an essential signal molecule and functional part of the DNA regeneration processes in the activated glial cells of the brain, which remains to be determined in more detail.

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